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# COMMENTARY

## ORAL CONTRACEPTIVES—POSSIBLE MEDIATION OF SIDE EFFECTS VIA AN ESTROGEN RECEPTOR IN LIVER*

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*Estrogens in oral contraceptives—benefits and side effects*

The combined oral contraceptives contain an estrogen (17-alpha ethinyl estradiol or mestranol) and a progestin. This combined pill is the most effective method of birth control currently available [1]. Users of the combined oral contraceptive have a lower unwanted pregnancy rate and less breakthrough bleeding than users of the oral contraceptive containing only a progestin (minipill). The lower pregnancy rate of the combined pill is in part due to an estrogen action at the hypothalamic-pituitary axis. FSH secretion is reduced and the ovarian follicles do not mature [2]. The estrogen also acts at the endometrium to prevent irregular shedding of cells which leads to bleeding between periods. Accordingly, the inclusion of the estrogen provides substantial benefits; however, there are also deficits.

Rare, but serious, side effects associated with the combined oral contraceptives have been related to the presence of the estrogen. The estrogen may contribute to the observed increase in the incidence of thrombosis, heart attacks, hypertension, gallbladder disease and liver tumors [3]. The increase in incidence of heart attacks is most marked in women older than 40 years of age who are still taking the birth control pill and who also smoke cigarettes [4]. The rare liver tumors are usually benign, but a few fatalities have been reported following hemorrhage into the liver and peritoneal cavity [5]. For women in their earlier reproductive years, the mortality from the pill is low. It is lower than deaths related to unwanted pregnancies if no contraceptives are used and is in the same range as the mortality due to other single, reversible methods of birth control (considering deaths due to the method and to fatal complications of unwanted pregnancies if the method fails) [6].

Estrogens have been used alone in some other clinical situations. Estrogen replacement therapy during and after menopause has been associated with an increased risk of gallbladder disease [7], hypertension [8] and the detection of endometrial cancer [9]. There has also been one report of a hepatoma found during estrogen therapy (which regressed after discontinuing the estrogen) [10]. An increase in thromboembolism was detected in therapeutic trials when high doses of estrogens were administered to men either with prostatic cancer [11] or after a heart attack [12].

*Possible mechanism of estrogen-mediated side effects—via the liver?*

The mechanisms by which estrogens increase the incidence of the side effects are not established. The side effects might be produced by the estrogens acting in multiple organs and by several separate mechanisms. At least some of the major side effects may be initiated by the interaction of an estrogen with the liver, producing changes in liver function which may then contribute to a side effect in a susceptible woman.

The liver is likely to be the estrogen target organ for the observed increase in the incidence of gallstones and of the hepatomas. It has been shown that women using the combined oral contraceptives have an increased concentration of cholesterol relative to bile acid in the bile secreted by the liver. The cholesterol, which is normally kept in colloidal solution by the bile acids, is supersaturated in concentration and precipitates as stones in the gallbladder [13].

Although the blood vessels are ultimately involved in the cardiovascular complications, it is not known if this is a direct or indirect estrogen effect. One report has described vascular lesions thought to have some distinctive features in oral contraceptive users with fatal thromboses [14]. Indirect influences on blood vessels could be exerted by changes in clotting mechanisms, platelet function, atherogenesis and hypertension. The levels of plasma clotting factors [15] and inhibitors [16], lipoproteins [17] and renin substrate [18] are changed in women taking oral contraceptives. These plasma proteins are synthesized in the liver.

An increase in clotting factors VII and X [15] and a decrease in the clotting inhibitor anti-thrombin III [16] may contribute to the enhanced occurrence of thrombosis [17]. Estrogens might contribute to heart attacks both by the clotting abnormalities and by accelerating atherosclerosis via enhanced hepatic synthesis of plasma triglycerides and prebeta-lipoproteins [17]. Elevated levels of plasma renin substrate observed in oral contraceptive users might initiate the development of hypertension. Renin substrate is cleaved by the enzyme renin from the kidney into angiotensin which is rapidly converted to the potent vasoconstrictor antigiotensin II. An increase in angiotensin resulting from elevated plasma renin substrate usually has a feedback action at the kidney to inhibit renin secretion. An explanation for individual susceptibility to the development of hypertension while using the oral contra-

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ceptives has been proposed [18]. In the steady state, most oral contraceptive users have high renin substrate, low renin and normal angiotensin levels. Women taking the pill who become hypertensive do not have a normal negative feedback of angiotensin on kidney renin secretion and elevated levels of angiotensin are maintained [18]. The metabolic changes mentioned and some of the side effects (e.g. many cases of hypertension) are reversible upon discontinuation of the oral contraceptive.

As a unifying concept (which simplifies estrogen interaction to a single organ and might explain the observed metabolic changes), it is possible that most of the oral contraceptive side effects are due to an estrogen interaction in the liver. The estrogen may change liver function including the synthesis of critical plasma proteins that influence the cardiovascular system.

*Estrogen receptor in the liver*

Changes in mammalian liver function after estrogen administration have been noted over the past decade [19, 20]. However, it has not been established whether the modifications in liver function are due to a direct or indirect estrogen–liver interaction. Estrogens might change the secretion of other organs and these secretions then act at the liver. For example, estrogens act at the pituitary to modify the secretion of pituitary hormones. It is conceivable that the liver is responding to altered levels of pituitary hormones. If the interaction of estrogen with liver is direct, it is likely that an estrogen receptor would be required.

Although putative estrogen receptors were easily demonstrated in target organs such as the rat uterus and pituitary, early attempts to demonstrate an estrogen receptor in the mammalian liver were unsuccessful. In these early studies, liver preparations from immature female rats were used to avoid the possibility that endogenous estrogen, secreted by the mature ovary, might have occupied the estrogen receptors. This laboratory found that estrogen binding was readily detectable when the liver cytosol was prepared from adult female rats [21–24]. The liver estrogen receptor, unlike that described in any other organ to date, increases 5- to 10-fold at about the time of puberty in the rat. The concentration of estrogen receptors in the adult female rat liver is approximately ⅓ of the concentration found in uterine cytosol. Assuming that one estrogen molecule binds to each receptor, the number of estrogen receptors is estimated to be 6200/hepatocyte. The liver receptors have been shown to bind estrogens with high specificity and to consist, at least in part, of protein. The equilibrium dissociation constant for radioactive estradiol is $1 \times 10^{-10}$ M, indicating a strong binding affinity. An estrogen-specific binding protein has been found in the liver cytosol of all five mammalian species studied including the monkey.

The estrogen-specific binding sites in the mammalian liver appear to be receptors since they fulfill several aspects of the current concept of steroid hormone action. It is thought that steroid hormones first bind to cytoplasmic receptors. The cytoplasmic receptor–steroid complex then moves from the cytoplasm to the nucleus (translocation) and attaches to chromatin. *In vitro*, the translocation is a temperature-dependent step [25, 26]. We have demonstrated translocation in the rat liver. After short periods of incubation of adult female rat liver slices with radioactive estradiol, initial binding in cytosol was detected. After 30-min incubations at 25 or 37° (but not at 0°) the binding protein is no longer detectable in the cytoplasm, but can be found in highly purified nuclei (sedimented through dense sucrose). Both the initial cytosol and subsequent nuclear radioactive binding can be prevented by adding non-radioactive diethylstilbestrol (DES) to the slice incubation [27]. DES is a strong competitor for radioactive estradiol binding to cytoplasmic estrogen receptors. Estrogen binding and translocation in rat liver also can be shown *in vivo*. After administration of an estrogen, the concentration of cytoplasmic receptor is diminished. A concomitant increase in the estrogen–receptor complex in highly purified nuclei is observed. The estrogen specificity of the receptors which appear in the nuclei is similar to the specificity of the receptor initially present in the cytoplasm [28].

The increase in estrogen binding in the liver accompanying sexual maturation of the rat correlates with a specific response to the administration of estrogens. The estrogen response of greatest relative magnitude in the rat liver is an increase in the synthesis of the plasma protein renin substrate. Administration of 100 μg ethinyl estradiol for 2 days increases plasma renin substrate 169 per cent above control levels in the adult but has little effect on the plasma renin substrate in the prepubescent rat [27]. The increase in plasma renin substrate in the adult rat has previously been shown to be due to increased liver synthesis of the protein and is a direct liver effect of the estrogen. Addition of DES to an isolated perfused rat liver preparation increases the synthesis and secretion of renin substrate into the perfusate [29]. In contrast to estrogen administration, prepubescent rats exhibit the same increase in plasma renin substrate as the adult rat after glucocorticoid administration. This preferential absence of response to estrogens is consistent with a low concentration of estrogen receptors in the prepubescent rat liver.

*Potential dissociation of estrogen effects in liver from effects in other target organs*

If some of the major side effects are mediated by a receptor–estrogen interaction in liver, the safety of the oral contraceptives might be improved by minimizing this liver interaction. At the same time, in order to derive the benefits of the estrogen, the effects in the hypothalamic-pituitary axis and in the endometrium would have to be maintained. Thus, a preferential diminution in liver estrogen–receptor interaction or function might lead to estrogenic action with an improved therapeutic ratio. This might be accomplished by utilizing some difference in the estrogen–receptor function in the liver from the estrogen–receptor function in the hypothalamus, pituitary and uterus.

Figure 1 represents a simplified schematic sequence of the events which are considered to constitute the process of steroid–receptor function.

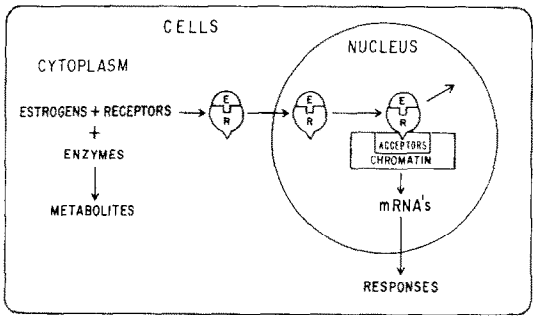


Fig. 1. Estrogen–receptor function in target cells. Key: E represents estrogens and R represents the receptors. Initial cellular changes are symbolized by messenger RNAs.

The cell under consideration is a variable; it could represent a hepatocyte, endometrial cell or pituitary cell. The chemical identity of the estrogen is also a variable; for example, it could be ethinyl estradiol or DES.

Theoretically the estrogen receptors in various cells could be different proteins. Thus far, there is no experimental evidence suggesting any substantial difference in the estrogen binding characteristics of the cytoplasmic receptor of the liver and the cytoplasmic receptor of the uterus. However, there seems to be a quantitative difference in the number of receptors per cell. The number of estrogen receptors determined *in vitro* per hepatocyte is lower than the number of receptors per uterine cell [28] or pituitary cell [30]. Estimating from autoradiographic localization studies *in vivo*, the hypothalamic neurons which concentrate estradiol also seem to contain high levels of receptor [31].

A second difference is that the liver is the site of extensive metabolism of steroids including estrogens to inactive derivatives. In liver slices, radioactive estradiol is rapidly converted to metabolites. Most of the metabolites do not seem to bind to the cytoplasmic receptor and promote its translocation to the nucleus [27]. Accordingly, the free concentration of a potent estrogen may be lower in the cytoplasm of a hepatocyte than in the cytoplasm of an endometrial or pituitary cell or in a hypothalamic neuron. Since the rate of receptor–estrogen complex formation is second order, a low concentration of receptor multiplied by a low concentration of potent estrogen in cytoplasm may result in the formation of receptor–estrogen complexes at a lower rate in liver relative to the rate in other target cells. We have observed that, 60 min after subcutaneous administration of 5 $\mu$g ethinyl estradiol to adult female rats, the receptor in the uterus is depleted from the cytoplasm and is found in the nucleus while only a small fraction of the receptor in the liver is translocated. The number of receptor–estrogen complexes in the nucleus is then estimated to be 800/liver cell and 8000/uterine cell. Only at higher doses of ethinyl estradiol (e.g. 100 $\mu$g) is a substantial portion of the cytoplasmic receptor in liver translocated to the nucleus [28].

A third and more speculative possibility is that there may be differences among cell types at steps subsequent to the translocation of the estrogen–receptor complex to the nucleus. In the nucleus it is postulated that the complex interacts with specific acceptors (acidic non-histone proteins?) on the chromatin. These multiple acceptors may regulate activity at different genes and lead to the variety of responses. The characteristics of the binding of the receptor–estrogen complex may vary at the chromatin acceptors among different cells and even for the variety of chromatin acceptors responsible for multiple effects in the same cell. It is proposed that the steroid–receptor complexes may change the attachment of RNA polymerase to initiation sites on the genes and may regulate the synthesis of selective messenger RNA molecules [15]. The quantitative and temporal relationships between binding of the receptor–estrogen complexes to the chromatin acceptors and the initial responses (e.g. mRNA synthesis) may also be highly variable. It is known that more cytoplasmic receptor–estrogen complexes translocate to the nucleus shortly after estrogen administration than are retained for several hours. The mechanisms for short- and long-term disposal of the complexes in the nucleus are not known.

The following examples illustrate what may be differences in receptor–estrogen nuclear acceptor interaction and responses. After subcutaneous injection of estrogens in saline to rats, estriol is as efficacious (same maximal effect) as estradiol on the uterus with respect to early cellular responses (water uptake, glucose oxidation) but much less efficacious with respect to a later response (increase in dry weight). This seems to be related to the brief retention in the nucleus of the receptor–estriol complex, while some of the receptor–estradiol complexes are retained for 12 hr [32]. Estriol is as effective as estradiol in maintaining the receptor in the nucleus and in promoting an increase in dry weight if administration is continuous (from paraffin pellet implants) [33]. Some triphenylethylene derivatives including nafoxidine, clomiphene and tamoxifen have been described as anti-estrogens. These drugs appear to attach to the estrogen cytoplasmic receptor and to translocate as a complex to the nucleus. However, the interaction in the nucleus seems to be different from that of the receptor–estradiol complex. A greater fraction of the receptor–anti-estrogen complexes can be extracted with 0.3 M KCl from uterine nuclei [34]. After administration *in vivo* the anti-estrogen–receptor complexes may be partially retained in the nucleus for as long as 19 days after a single injection [35]. The term anti-estrogen is an inadequate description for these drugs. They can produce some uterine effects as well as estradiol (e.g. increase in uterine weight after a single injection) while they may not produce some other effects. Translocation of the receptor–estradiol complex to the nucleus leads to protein synthesis of new estrogen cytoplasmic receptors (replenishment). Replenishment after anti-estrogens may be long delayed and might partially explain the refractory state observed to subsequent administration of estradiol [36]. Anti-estrogens may also have complicated effects on the liver. For example, administration of the estrogen ethinyl estradiol to monkeys increases plasma transcortin and thyroxine binding globulin and decreases plasma haptoglobin, most

likely by changing their hepatic synthesis. Nafoxidine administration increases transcortin and decreases haptoglobin but does not increase thyroxine binding globulin. The increase in thyroxine binding globulin with ethinyl estradiol can be blocked by coadministration of nafoxidine [37]. Thus, nafoxidine behaves as an agonist with respect to transcortin and haptoglobin and an estrogen antagonist with respect to thyroxine binding globulin regulation. Perhaps the nafoxidine–receptor complex in liver chromatin is effective at the acceptors that increase mRNA for transcortin and decrease mRNA for haptoglobin but does not bind or is ineffective at the acceptor regulating mRNA for thyroxine binding globulin. This model suggests that the estrogen receptor can exist in different conformations in the nucleus depending upon the drug attached, with variable binding to acceptors and response characteristics.

Based upon the model presented, estrogen responses in liver might differ from estrogen responses in other target organs due to relatively lower concentrations of receptor–estrogen complexes translocated to the nucleus or to the interaction or function of these complexes with the genome.

The receptor studies in the rat liver suggest that at least some direct estrogen mediated effects on liver should require higher doses of estrogen than are required for effects in other organs. A high dose (300 $\mu$g) of ethinyl estradiol is required to obtain a maximal increase in plasma renin substrate of rats 24 hr later [38]. In contrast, only a few $\mu$g of estradiol or ethinyl estradiol are required for maximal uterotrophic effects. The doses of estrogens that elevate plasma transcortin are also relatively high in the human. The doses of estrogens for half-maximal increase in transcortin (administered daily for 2 weeks to men) are 90 $\mu$g ethinyl estradiol, 1.3 mg DES or 35 mg premarin [39]. The dose of ethinyl estradiol recommended by the FDA for combined oral contraceptives is 50 $\mu$g or less.

*Current and potential modifications of the combined oral contraceptives*

The newer combined oral contraceptives contain lower amounts of both the estrogen, ethinyl estradiol, and of the progestin. Decreasing the amount of the ethinyl estradiol to about 30 $\mu$g seems to retain contraceptive effectiveness (and to produce regular menstrual bleeding patterns in most women). Some estrogen related plasma protein changes are observed with 30 $\mu$g ethinyl estradiol in the combined pill, but the changes are less than with higher doses of the estrogen [40]. The only evidence to date that reducing the ethinyl estradiol dose diminishes a major side effect is that pills containing 50 $\mu$g increase the incidence of thrombosis less than do preparations containing more than 50 $\mu$g [41, 42].

It is not yet known whether decreasing the ethinyl estradiol dose below 50 $\mu$g will further reduce the risk of thrombosis or of the other side effects. An ongoing large scale prospective study of oral contraceptive users and non-users has recently indicated that an increased risk of cardiovascular disease is still observed in oral contraceptive users, but insufficient information is available to assess the value of reducing the ethinyl estradiol dose below 50 $\mu$g [43].

Alternatively, a safer combined oral contraceptive might be obtained by changing the estrogen. If the major side effects are mediated by estrogen–receptor interactions in the liver, another estrogen could be selected or developed which preferentially minimized the liver–estrogen interaction or function while producing estrogen effects in the hypothalamic-pituitary axis and in the endometrium.

*Summary*

An estrogen receptor has been demonstrated in the mammalian liver. It is a cytoplasmic protein with a high specificity and affinity for binding estrogens. The receptor–estrogen complex can translocate to the nucleus of the liver cell. At least some of the major side effects of the estrogens in oral contraceptives (and in preparations used at menopause) may be due to the estrogen directly acting at the liver. The receptor–estrogen complex may modify hepatic function including changing the synthesis of some critical plasma proteins. Estrogen–receptor function in liver may differ from that in other target organs. This difference might provide a molecular basis for the possibility of diminishing side effects while maintaining the desired effects. It might provide a basis for the possibility that the newer combined oral contraceptives with lower amounts of ethinyl estradiol will have less side effects. Alternatively, further elucidation of the function of the estrogen receptor in the mammalian liver might lead to the design or selection of a safer estrogen.

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